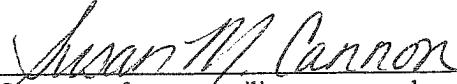


PATENT
ATTORNEY DOCKET NO. 00786/381002

Certificate of Mailing: Date of Deposit: December 4, 2002

I hereby certify under 37 C.F.R. § 1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated above and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

Susan M. Cannon
Printed name of person mailing correspondence


Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Mark C. Fishman et al.

Art Unit: 1634

Serial No.: 09/759,508

Examiner: Jehanne E. Souaya

Filed: January 12, 2001

Customer No.: 21559

Title: Methods for Diagnosing and Treating Heart Disease

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF XIAOLEI XU, PH.D., UNDER 37 C.F.R. § 1.131

I declare:

1. I am an inventor of the subject matter that is described and claimed in the above-captioned patent application.
2. The enclosed Exhibit is a copy of pages from my laboratory notebook, which show that my co-inventor and I had determined that the pickwick mutation, which is characterized by a weak heartbeat, is in the titin gene. In particular, we found that certain zebrafish sequences that we had identified as being in the pickwick locus were homologous to known titin sequences. These pages are dated prior to the August, 1999 publication date of Satoh et al. (Biochem. Biophys. Res. Com. 262:411-417, 1999).

3. All statements made herein of my own knowledge are true, and all statements made on information and belief are believed to be true, and further, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 11/11/02


Xiaolei Xu
Xiaolei Xu, Ph.D.
14 Wait Street, Apartment 2R
Boston, MA 02120

Pickwickpositional cloning

LG 9

100% never use more than 1 well.

5cm

10789. (8 RE / 1,000)

connection

10m.

1 YAC

500 embryo

3 YAC.

by primers

from 3 intr of connexin

as superpool.

II were on V₁₀ use the primers pair to screen the 8 plate pool

Total 24 PCR reaction.

p2
8
28. 30 * 3 = 90.Genomic DNA 1 675 50X. 3 ~~2~~ / 2 use 32

order	COS	envelope	35 cycle	x90
25A.	Temp plate	4 A	(0.018/2)	26.
	10K A	2.5 A		22.5
17.8	25mM dNTP	0.1 A		9
90				
1602	primer	0.25 A	20 mM	22.5
	- primer	0.5		22.5
Tag		0.1 A		9
H2O 4L		17.8 A		1602
		25 A		

1. IVF. fish do not synchronize our eggs try
next week. select strong fish!
try with my n836 wt fish although AB/T4
background. at least. get something.

2.

Titin pickwick could be within. zeb256 show high homology with
titin (connectin), which makes sense.

1. Z 8363 scan all embryos to identify recombinants.

> 500 embryos. confirm with z 20031 either and ID'd.

2. design primers from af 036148. do ~~RT-PCR~~ PCR

together with zeb256 against Y5, Y6. hope to pick

up the right side about Y5T3, Y6T3

3. Compare human, mouse 100kb / titin sequence. design
primer pairs against the 27 kb cDNA (conserved region)

① put into RH map. confirm its identity.

② PCR against Y5, Y6.

③ isolate BAC & get the insertion. 3'UTR region

and then design primers for SSCP.

~~Design primers~~

1. Got embryos for m1062H (their parents are here)

m686.g x TL⁰² (two pairs)

m1010H

m521A (# are low)

mP18a (TL allele)

Today bleached five out of 6 except m521A
next Tuesday put them into system.

Tomorrow look at phenotype

More good news!

1. from EST project. If were titin zebrafish version!

2. Y5T7 end ~~titin~~ is titin homologue!

3. Best of all. they represent different positions of titin

2.9K	3.3	5.2	24	26	26.5	2
AJ58854	AJ601282					
T1	AJ588106	T3				
	T2					

4 according to sequence alignment of Y5T7 the titin gene in chromosome should be

z8363

0

z661256

z4K1

z7K

+titin

Y5T3 ← -

Y5T7